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High glucose inhibits glucose uptake in renal proximal tubule cells by oxidative stress and protein kinase C

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High glucose inhibits glucose uptake in renal proximal tubule cells by oxidative stress and protein kinase C.

Background. High glucose has been considered to play an important role in alteration of renal proximal tubule transporter's activity. This study examined the mechanism by which high glucose modulates α-methyl-D-glucopyranoside (α-MG) uptake in primary cultured rabbit renal proximal tubule cells (PTCs).

Methods. PTCs were incubated with 25 mmol/L glucose alone or combined with taurine, ascorbic acid, catalase, staurosporine, and bisindolylmaleimide I. Then α-MG uptake and lipid peroxide (LPO) formation were examined.

Results. Twenty-five mmol/L glucose from four hours, but not 25 mmol/L mannitol, inhibited α-MG uptake by 23% compared with 5 mmol/L glucose (control). In the study to examine the relationship of oxidative stress in the high-glucose–induced inhibition of α-MG uptake, 25 mmol/L glucose significantly increased LPO by 27% compared with control. However, 10 mmol/L glucose did not affect α-MG uptake and LPO formation. Taurine (2 mmol/L), ascorbic acid (1 mmol/L), endogenous antioxidants, or catalase (600 U/mL) significantly blocked 25 mmol/L glucose-induced increase of LPO formation and inhibition of α-MG uptake. In the experiment to examine the effects of protein kinase C on LPO formation, 12-O-tetradecanoylphorbol-13-acetate (TPA; 100 ng/mL) increased LPO formation, and staurosporine (10^{-7} mol/L) and bisindolylmaleimide I (10^{-6} mol/L) totally blocked 25 mmol/L glucose-induced increase of LPO formation and inhibition of α-MG uptake. In addition, taurine reduced TPA-induced increase of LPO formation and inhibition of α-MG uptake.

Conclusion. High glucose induces, in part, the inhibition of α-MG uptake through LPO formation, and activation of protein kinase C may play a role in high-glucose–induced LPO formation in the primary cultured rabbit renal PTCs.

Hyperglycemia is a major etiologic factor in the complications of diabetes, including nephropathy [1, 2]. An elevated glucose concentration may lead to an increase in reactive oxygen species production, as well as to the attenuation of free radical scavenging molecules [3]. It has been widely accepted that free radicals play an important role in the pathogenesis of diabetic nephropathy by their severe cytotoxic effects, such as lipid peroxidation and protein denaturation in cell membrane, followed by the alteration of the membrane fluidity, enzyme properties, and ion transports [4, 5]. In vitro, high ambient glucose can cause lipid peroxidation in isolated rat glomeruli. A one-year study in streptozotocin (STZ)-diabetic rats has shown that dietary supplementation with the antioxidant taurine ameliorates the functional and structural manifestations of diabetic nephropathy [6, 7]. However, there have been very few reports concerning the functional alteration of renal proximal tubule transporter's activity in diabetic nephropathy.

Two types of glucose transporters have been identified in higher organism: Na^+-coupled glucose transporters (SGLT1-2), which couples glucose uptake to the inwardly directed electrochemical gradient, and facilitated glucose transporters (GLUT1-5), which permit passive movement of glucose across the plasma membranes down its concentration gradient [8–11]. A decrease in SGLTs activity has been reported in brush border membrane vesicles of diabetic rats, indicating that intrinsic change has occurred in SGLTs in the plasma membrane. A direct involvement of glucose in the inhibition of the SGLTs has been indicated in studies of LLC-PK_1 cells [12]. Moran, Turner, and Handler showed that prolonged incubations of LLC-PK_1 cells with high glucose resulted in a reduction in SGLTs activity, as well as the number of SGLTs [12]. A recent report showed that oxidant altered SGLTs in LLC-PK_1 cells [13]. Glucose is the major energy source for most mammalian cells and provides much of the mass for macromolecular synthesis, whereas excessive glucose can be transported intracellularly and metabolized to change redox potential, increase sorbitol production via aldose reductase, or alter signal transduction pathways, such as the activation of diacylglycerol (DAG) and protein kinase C (PKC) levels [14–16]. In rat renal glomeru-

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lus, high glucose activated PKC [15]. However, the mechanisms by which the SGLTs' activity is decreased in proximal tubule in diabetes are poorly understood, although hyperglycemia has been proposed as an underlying cause.

The primary cultured rabbit renal proximal tubule cells (PTCs) culture system that was used in this study has been well recognized in vitro to retain the differentiated phenotype typical of the renal proximal tubule, including a polarized morphology [17], distinctive proximal tubule transport systems (the Na/glucose cotransport system [18] and the p-aminohippurate transport system [19]), and hormone responses. PTCs have a parathyroid hormone (PTH)-sensitive adenylate cyclase-like observation *in vivo* in the renal PTCs [18]. Responsiveness to PTH is not only observed in the renal cortex, but arginine vasopressin has a similar effect on cAMP production, caused by a small number of distal tubule cells in this preparation [18]. However, the PTCs do not similarly respond to arginine vasopressin by producing cAMP, indicating this culture system is highly purified with respect to renal PTCs [18]. Therefore, PTCs in hormonally defined, serum-free culture conditions would be a powerful tool for studying the effect of high glucose on renal glucose transport. To investigate the mechanism by which high glucose modulates α-MG uptake of PTCs, we examined the involvement of oxidative stress, and furthermore, the involvement of PKC in this high-glucose–induced oxidative stress.

METHODS

Isolation of rabbit renal proximal tubules and culture conditions

Primary rabbit renal PTC cultures were prepared by the method of Chung et al [18]. The PTC was grown in Dulbecco's modified Eagle's medium (DMEM)/F-12 medium with 15 mmol/L HEPES and 20 mmol/L sodium bicarbonate (pH 7.4). Immediately prior to the use of the medium, three growth supplements (5 μg/mL insulin, 5 μg/mL transferrin, and 5×10^{-5} mol/L hydrocortisone) were added. The kidneys of a rabbit were perfused via the renal artery, first with phosphate-buffered saline (PBS) and then with medium containing 0.5% iron oxide. Renal cortical slices were prepared and homogenized. The homogenate was poured first through a 253 μm and then an 83 μm mesh filter. Tubules and glomeruli on top of the 83 μm filter were transferred into sterile medium. Glomeruli (containing iron oxide) were removed with the stirring bar. The remaining tubules were briefly incubated in medium. The tubules were then washed by centrifugation, resuspended in medium containing the three supplements, and transferred into tissue culture dishes. Medium was changed one day after plating and every two days thereafter. PTCs were maintained in a 37°C, 5% CO_2 humidified environment in a serum-free basal medium supplemented with three growth supplements.

Uptake experiments

To study effect of high glucose on α-MG uptake, the confluent monolayers were incubated with 10, 25, or 50 mmol/L (final concentration) glucose for four hours before ^{14}C-α-MG uptake. α-MG uptake experiments were conducted as described by the method of Sakhrani et al [20]. To study α-MG uptake, the culture medium was removed by aspiration, and the monolayers were gently washed twice with the uptake buffer [136 mmol/L NaCl, 5.4 mmol/L KC1, 0.41 mmol/L $MgSO_4$, 1.3 mmol/L $CaCl_2$, 0.44 mmol/L Na_2HPO_4, 0.44 mmol/L KH_2PO_4, 5 mmol/L HEPES, 2 mmol/L glutamine, and 0.5 μg/μL bovine serum albumin (BSA), pH 7.4]. After the washing procedure, the monolayers were incubated at 37°C for 30 minutes in an uptake buffer that contained 0.5 mmol/L α-MG and ^{14}C-α-MG (0.5 μCi/mL). At the end of the incubation period, the monolayers were again washed three times with ice-cold uptake buffer, and the cells were solubilized in 0.1% sodium dodecyl sulfate (SDS; 1 mL). To determine the ^{14}C-α-MG incorporated intracellulary, 900 μL of each sample were removed and counted in a liquid scintillation counter (Beckmann Co., Palo Alto, CA, USA). The remainder of each sample was used for protein determination. The protein content of each sample was determined by the Bradford method [21]. The radioactivity counts in each sample were then normalized with respect to protein and were corrected for zero-time uptake per mg protein. All uptake measurements were made in triplicate.

Na^+ uptake experiment was conducted as described by the method of Rindler, Taub, and Saier [22] and Pi uptake experiment as described by the method of Rabito et al [23]. 3-O-MG uptake was conducted using [^{14}C]-3-O-MG instead of [^{14}C]-α-MG. The next steps were conducted as described in α-MG uptake.

Lactate dehydrogenase assay

Cell injury was assessed by lactate dehydrogenase (LDH) activity. The level of LDH activity in the medium was measured by using a LDH assay kit. For measurement of LDH activity, PTCs were treated with 10, 25, or 50 mmol/L glucose for four hours. LDH activity was expressed as Wroblewski units per microgram of protein.

Trypan blue exclusion assay

Proximal tubule cells were grown to confluence in 35 mm dishes, as described earlier in this article. For measurement of trypan blue exclusion assay, PTCs were treated with 10, 25, or 50 mmol/L glucose for four hours. Monolayers were washed twice with PBS. The cells were detached from the culture dishes using 0.05% trypsin/

0.5 mmol/L ethylenediaminetetraacetic (EDTA) acid solution, and proteolytic action was then inhibited by soybean trypsin inhibitor (0.05 mg/mL). Then 0.4% (wt/vol) trypan blue solution (500 μL) was added to the cell suspension, and the cells were counted, keeping a separate count of blue cells, using a hemocytometer under light microscopy. Cells failing to exclude the dye were considered nonviable, and the data are expressed as percentage of viable cells.

Measurement of lipid peroxides

The levels of lipid peroxides (LPO) in the monolayer cells was determined by measuring malondealdehyde content according to the method of Ohkawa, Ohishi, and Yagi [24]. Monolayers were washed twice with PBS, and the cells were harvested, sonicated. One hundred microliters of sonicated cells were mixed with 8% SDS (100 μL), 0.8% 2-thiobarbituric acid (TBA; 200 μL), and 20% acetic acid (200 μL). The mixture was heated to 95°C for 60 minutes. After reaction time, this mixture was cooled in ice-cold water. To extract nonspecific red pigment, n-butanol-pyridine mixture (15:1 vol/vol, 1 mL) added, the mixture was shaken vigorously, and centrifuged at 4000 r.p.m. for 10 minutes. The upper organic layer was measured by spectrofluorometry at emission wavelength 553 nm with excitation wavelength 515 nm. 1,1,3,3-Tetraethoxypropane was used as a standard, and the values of LPO for samples were expressed as nmol/mg protein. In this study, taurine was added to cell mixtures to prevent any initiation of membrane lipid peroxidation during the assay. An addition of taurine to standard 1,1,3,3-tetraethoxypropane or sample did not affect its color development with the TBA (data not shown).

Chemicals

Adult New Zealand White male rabbits (1.5 to 2.0 kg) were purchased from Dae Han Experimental Animal Co, Ltd. (Chungju, Korea). Class IV collagenase and soybean trypsin inhibitor were purchased from Life Technologies (Grand Island, NY, USA). Staurosporine, D-glucose, mannitol, 12-O-tetradecanoylphorbol-13-acetate (TPA), phlorizin, 3-O-methyl-D-glucose (3-O-MG), hydrogen peroxide, 1,1,3,3-tetraethoxypropane, 2-TBA, trypan blue, ouabain, ascorbic acid, and catalse were obtained from Sigma Chemical Company (St. Louis, MO, USA). Bisindolylmaleimide I was purchased from Calbiochem (La Jolla, CA, USA). $^{22}Na^+$, ^{32}Pi, [^{14}C]-3-O-methyl-D-glucose, and [^{14}C]-α-methyl-D-glucopyranoside (α-MG) were purchased from Dupont/NEN (Boston, MA, USA). LDH assay kit was obtained Iatron Lab. (Tokyo, Japan), and all other reagents were of the highest purity commercially available. Liquiscint was obtained from National Diagnostics (Parsippany, NY, USA).

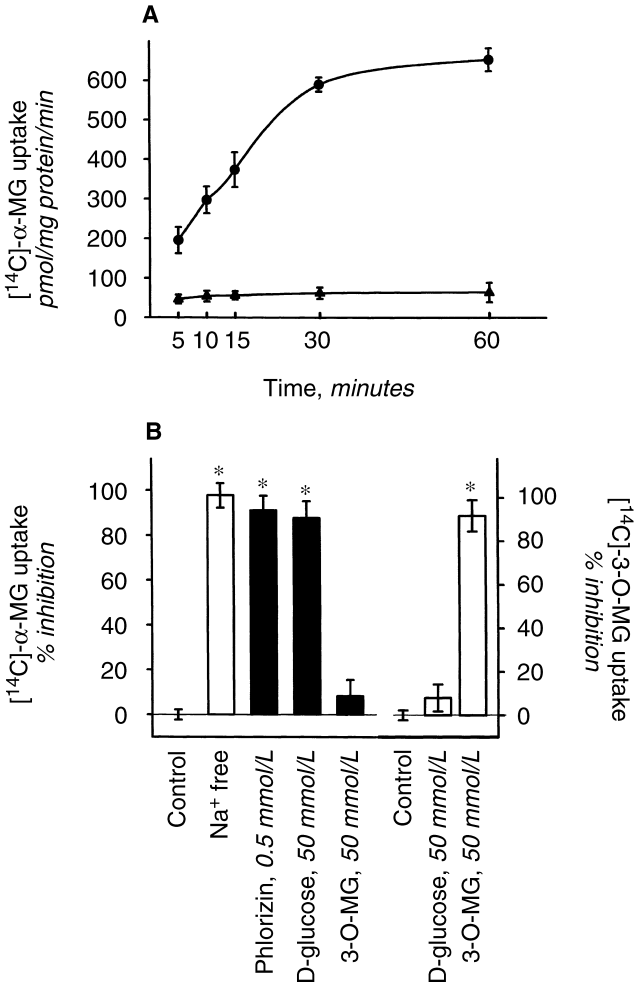

Fig. 1. Characterization of α-methyl-D-glucopyranoside (^{14}C-α-MG) uptake in proximal tubule cells. (*A*) α-MG uptake was measured in the presence of 136 mmol/L NaCl (●) or choline chloride (▲). (*B*) Effects of sodium, phlorizin, D-glucose, and 3-O-methyl-D-glucopyranoside (3-O-MG) on α-MG or 3-O-MG uptake. PTCs were treated with phlorizin (0.5 mmol/L), D-glucose (50 mmol/L), and 3-O-MG (50 mmol/L) for 30 minutes before the uptake experiment. NaCl was replaced by choline chloride. Values are the means ± SE of nine independent experiments with triplicate dishes. *$P < 0.05$ vs. control.

Statistical analysis

Results were expressed as means ± SE. Statistical analysis was performed using Student's *t*-test or analysis of variance. The difference was considered statistically significant when $P < 0.05$.

RESULTS

Characterization of α-methyl-D-glucopyranoside uptake in proximal tubule cells

We examined the time course in order to determine the "time point" of the uptake to be used for experiments. In this study, uptake was linear for 30 minutes (Fig. 1A). Accordingly, a 30-minute uptake was used in

all the experiments. As shown in Figure 1B, 0.5 mmol/L phlorizin inhibited α-MG uptake by more than 90%. Replacement of NaCl with choline chloride had an identical effect, in keeping with the known properties of glucose transporter in the apical membrane of the proximal tubules. In addition, experiments to examine the specificity of the glucose carrier showed that 50 mmol/L D-glucose significantly inhibited α-MG uptake, but 3-O-MG, a facilitated glucose transporter substrate in basolateral membrane of proximal tubule, did not. 3-O-MG uptake was also not affected by 50 mmol/L D-glucose.

Effect of high glucose on α-methyl-D-glucopyranoside uptake

To determine an effective concentration and treatment time for response of glucose on α-MG uptake, PTCs were incubated with different concentrations of glucose (10, 25, and 50 mmol/L) and with various times after initiation of the treatment. As shown in Figure 2, 25 and 50 mmol/L glucose for four hours significantly inhibited α-MG uptake by 23.16 ± 4.04% and 32.32 ± 4.08% compared with 5 mmol/L glucose (control), respectively. A statistically significant decrease in α-MG uptake was found after four hours from the start of the incubation (Fig. 2A). Prolonged incubation with high glucose for as long as 96 hours did not increase the inhibition. However, 10 mmol/L glucose did not affect α-MG uptake regardless of the treatment time.

High glucose concentrations can result in altered medium osmolarity. To rule out an influence of altered osmolarity on α-MG uptake, we also tested the effect of mannitol. α-MG uptake in monolayers treated with 10, 25, or 50 mmol/L mannitol for four hours did not cause any decrease (Fig. 2B). In addition, trypan blue exclusion and LDH, nonspecific cell injury marker enzyme, release studies to examine cell injury by 10, 25, or 50 mmol/L glucose treatment did not show significant changes at either control or high glucose levels (Table 1). Therefore, 25 and 50 mmol/L of glucose treatment for four hours were used as a high-glucose condition compared with 5 mmol/L glucose control in this study.

To examine the effect of high glucose on other brush border membrane transporters, PTCs were treated with vehicle, 10, 25, or 50 mmol/L glucose for four hours, and then ^{32}Pi and $^{22}Na^{+}$ uptakes were conducted. None of the glucose concentrations affected ^{32}Pi and $^{22}Na^{+}$ uptake (Table 2).

Role of oxidative stress in the high glucose-induced inhibition of α-methyl-D-glucopyranoside uptake

To examine relationship of oxidative stress in the high-glucose–induced inhibition of α-MG uptake, we measured the effect of high glucose on LPO formation. When cultured cells were preincubated for four hours with 10, 25, or 50 mmol/L glucose, 25 or 50 mmol/L glucose significantly increased LPO formation to 0.65 ± 0.03 or 0.69 ± 0.03 nmol/mg protein compared with control (0.51 ± 0.01 nmol/mg protein), respectively ($P < 0.05$; Fig. 3A). Cells were incubated with 10, 25, or 50 mmol/L glucose and with the different times after the initiation of the treatment LPO was measured. As shown in Figure 3A, a statistically significant increase in LPO was found at four hours after the start of the incubation with 25 mmol/L glucose. Prolonged incubation for as long as 96 hours did not change increase in LPO. However, 10 mmol/L glucose did not affect LPO formation. The oxidizing action of high glucose on cells was not caused by the hyperosmolarity of the medium because 25 and 50 mmol/L mannitol did not reproduce the effect of 25 and 50

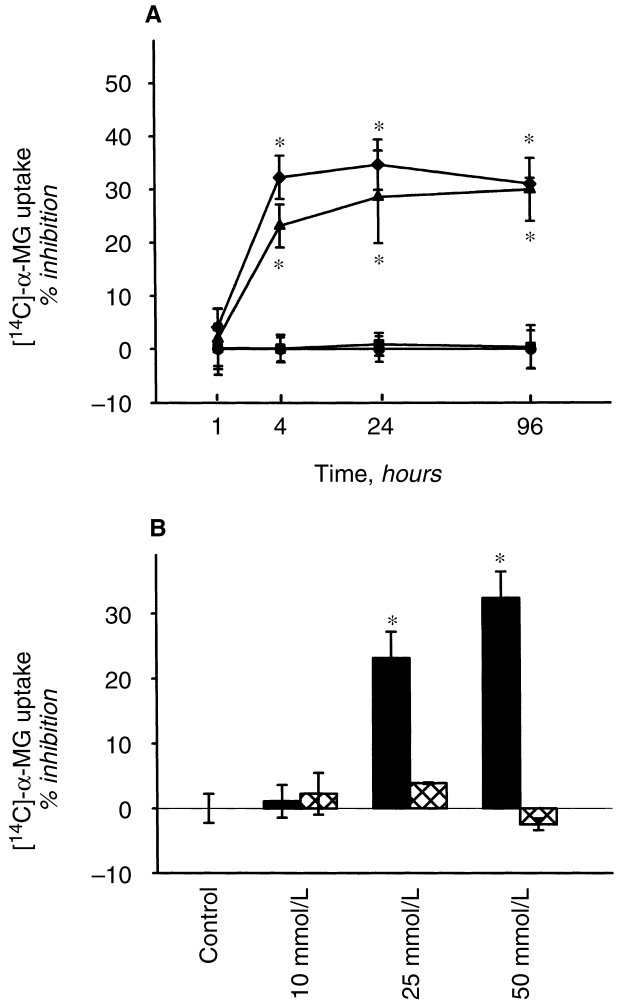

Fig. 2. Dose and time courses of high glucose on ^{14}C-α-MG uptake. (*A*) PTCs were treated with vehicle (●), 10 (■), 25 (▲), or 50 (◆) mmol/L glucose for 1 to 96 hours before the uptake experiment. The monolayer cells were incubated in uptake buffer containing ^{14}C-α-MG for 30 minutes at 37°C. (*B*) The percentage of glucose (■) and mannitol (⊠) inhibition of α-MG uptake after 10, 25, or 50 mmol/L treatment for four hours compared with control (□), respectively. Values are the means ± SE of four independent experiments with triplicate dishes. *$P < 0.05$ vs. control.

Table 1. Effects of high glucose on α-MG, Pi, and Na^+ uptake

	[^{14}C]-α-MG	^{32}Pi	$^{22}Na^+$
	pmol/mg protein/min		
Control	470.0 ± 10.6	250.0 ± 22.3	140.0 ± 3.6
10 mmol/L glucose	470.5 ± 5.1	256.2 ± 15.4	138.6 ± 4.3
25 mmol/L glucose	361.1 ± 10.4[a]	275.2 ± 28.3	144.1 ± 4.5
50 mmol/L glucose	319.6 ± 10.0[a]	214.1 ± 2.4	134.3 ± 0.4

Primary cultured renal proximal tubule cells were treated for four hours with 10, 25, or 50 mmol/L glucose. High glucose treated cells were then incubated with [^{14}C]-α-MG 0.5 μCi/mL, ^{32}Pi 1.5 μCi/mL, or $^{22}Na^+$ 0.25 μCi/mL for 30 minutes at 37°C. Uptake experiments were performed as described in the Methods section. Values are the means ± SE of four independent experiments with triplicate dishes.

[a] $P < 0.05$ vs. control

Table 2. Effects of high glucose on cell viability

	LDH *WU/100 μg protein*	Cell viability *% of control*
Control	28.9 ± 1.1	100.0 ± 1.7
10 mmol/L glucose	25.7 ± 2.2	98.8 ± 2.4
10 mmol/L mannitol	30.4 ± 3.2	102.4 ± 2.0
25 mmol/L glucose	31.6 ± 1.4	101.0 ± 1.2
25 mmol/L mannitol	27.5 ± 1.5	102.8 ± 0.7
50 mmol/L glucose	27.7 ± 1.4	101.0 ± 5.1
50 mmol/L mannitol	31.8 ± 2.9	101.8 ± 3.4

Primary cultured renal proximal tubule cells were treated with 10, 25, or 50 mmol/L glucose or mannitol for four hours, and then lactate dehydrogenase activity (LDH) assay and trypan blue exclusion experiments were conducted. Values are the means ± SE of three independent experiments with triplicate dishes.

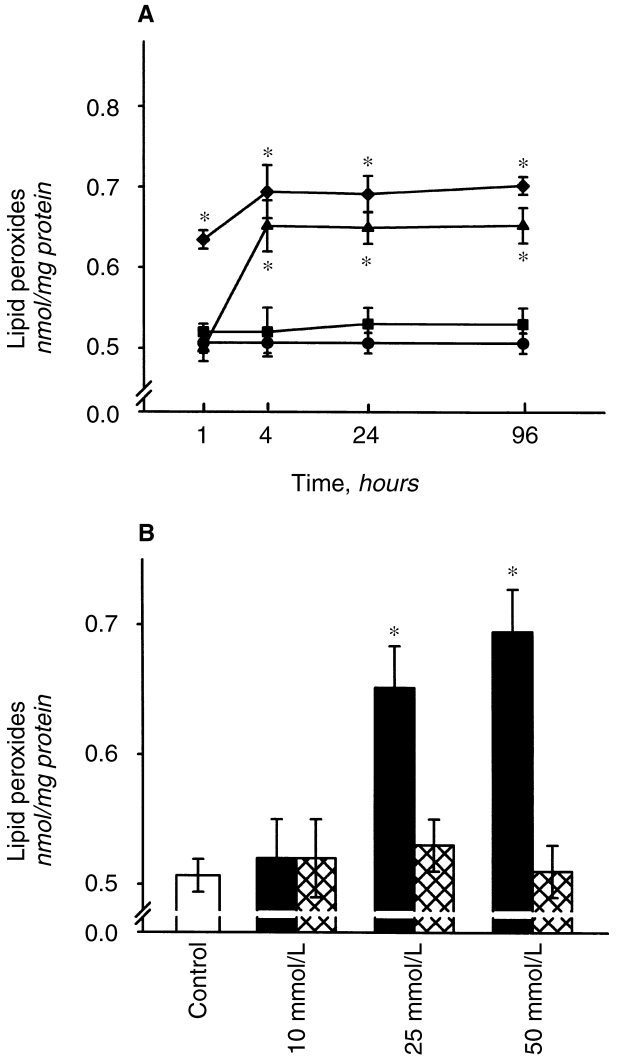

Fig. 3. Dose and time courses of high glucose on lipid peroxides (LPO) formation. (*A*) PTCs were treated with vehicle (●), 10 (■), 25 (▲), or 50 (◆) mmol/L glucose for 1 to 96 hours before LPO measurement. (*B*) LPO formation after 10, 25, or 50 mmol/L glucose (■) and mannitol (⊠) treatment for four hours compared with control (□), respectively. Values are the means ± SE of four independent experiments with triplicate dishes. *$P < 0.05$ vs. control.

mmol/Lglucose, respectively, on LPO formation (Fig. 3B). Figure 4 showed that 25 mmol/L glucose or H_2O_2 (10^{-7} mol/L) significantly increased LPO formation ($P < 0.05$), although there was no synergistic effect. These 25 mmol/L glucose-induced increases of LPO were effectively inhibited by addition of taurine (2 mmol/L), ascorbic acid (1 mmol/L), or catalase (600 U/mL), although taurine, ascorbic acid, or catalase alone did not cause any significant effect on LPO formation produced by 5 mmol/L glucose. Therefore, in order to examine role of oxidative stress in the 25 mmol/L glucose-induced inhibition of α-MG uptake H_2O_2, taurine, ascorbic acid, and catalase were used. As shown in Figure 5, H_2O_2 significantly inhibited α-MG uptake by 38.40 ± 6.44% compared with control ($P < 0.05$). However, taurine, ascorbic acid, or catalase prevented the inhibitory effect of 25 mmol/L glucose on α-MG uptake, although these itself had no significant effect on α-MG uptake.

Effects of protein kinase C on lipid peroxide formation and α-methyl-D-glucopyranoside uptake

To examine the effects of PKC on LPO formation and α-MG uptake, confluent monolayers were treated with 25 mmol/L glucose, TPA (100 ng/mL), an artificial PKC activator, or glucose in combination with TPA for four hours. Figure 6A showed that TPA alone and in combination with 25 mmol/L glucose increased LPO formation to 0.70 ± 0.80 or 0.86 ± 0.11 nmol/mg protein compared with control (0.48 ± 0.01 nmol/mg protein). Staurosporine (10^{-7} mol/L) and bisindolylmaleimide I (10^{-6} mol/L; PKC inhibitors) blocked 25 mmol/L glucose-induced stimulation of LPO formation. Therefore, we examined the relationship between oxidative stress and PKC in the 25 mmol/L glucose-induced inhibition of α-MG uptake. Figure 6B showed that TPA alone and in combination with 25 mmol/L glucose inhibited α-MG uptake (26.29 ± 4.15, 30.43 ± 3.87% compared with control). In addition, staurosporine and bisindolylmaleimide I totally blocked

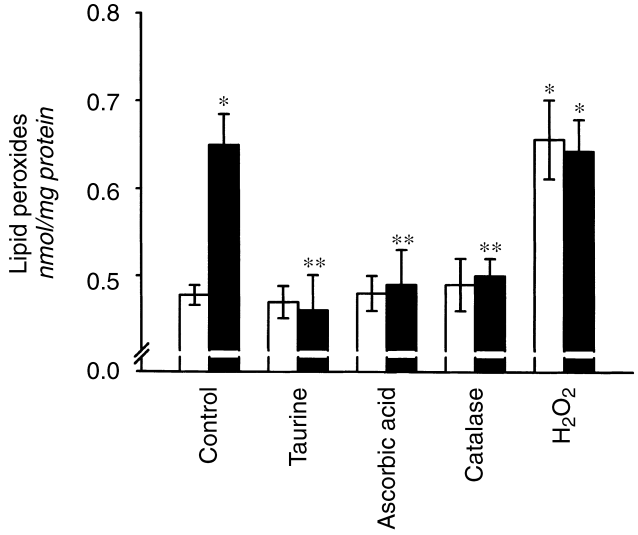

Fig. 4. Effects of antioxidants and hydrogen peroxide on 5 (□) or 25 (■) mmol/L glucose induced on lipid peroxides formation. PTCs were treated with taurine (2 mmol/L), ascorbic acid (1 mmol/L), and catalase (600 U/mL) for 30 minutes prior to the treatment of 25 mmol/L glucose for four hours or were incubated with 25 mmol/L glucose alone or together with hydrogen peroxide (10^{-7} mol/L) for four hours. Values are the means ± SE of four independent experiments with triplicate dishes. *$P < 0.05$ vs. control; **$P < 0.05$ vs. 25 mmol/L glucose alone.

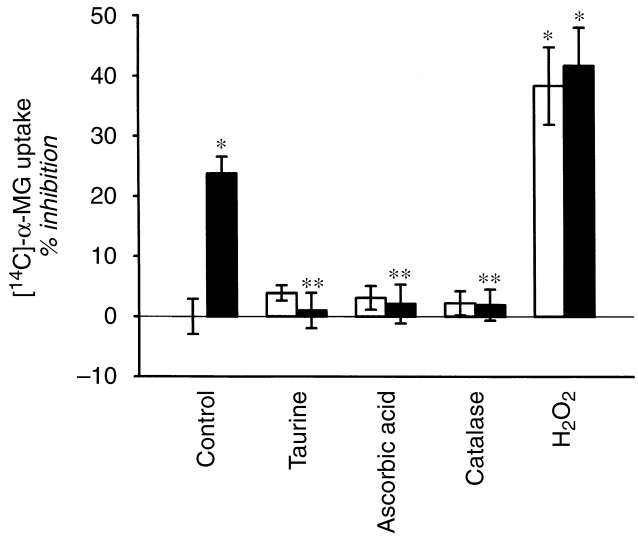

Fig. 5. Effects of antioxidants and hydrogen peroxide on 5 (□) or 25 (■) mmol/L glucose-induced on α-MG uptake. PTCs were treated with taurine (2 mmol/L), ascorbic acid (1 mmol/L), catalase (600 U/mL) for 30 minutes prior to the treatment of 25 mmol/L glucose for four hours or were incubated with 25 mmol/L glucose alone or together with hydrogen peroxide (10^{-7} mol/L) for four hours. Values are the means ± SE of four independent experiments with triplicate dishes. *$P < 0.05$ vs. control; **$P < 0.05$ vs. 25 mmol/L glucose alone.

25 mmol/L glucose-induced inhibition of α-MG uptake. In addition, in experiments to examine the effects of taurine on TPA-induced LPO formation and inhibition of α-MG uptake, these effects were significantly blocked by taurine (Fig. 7).

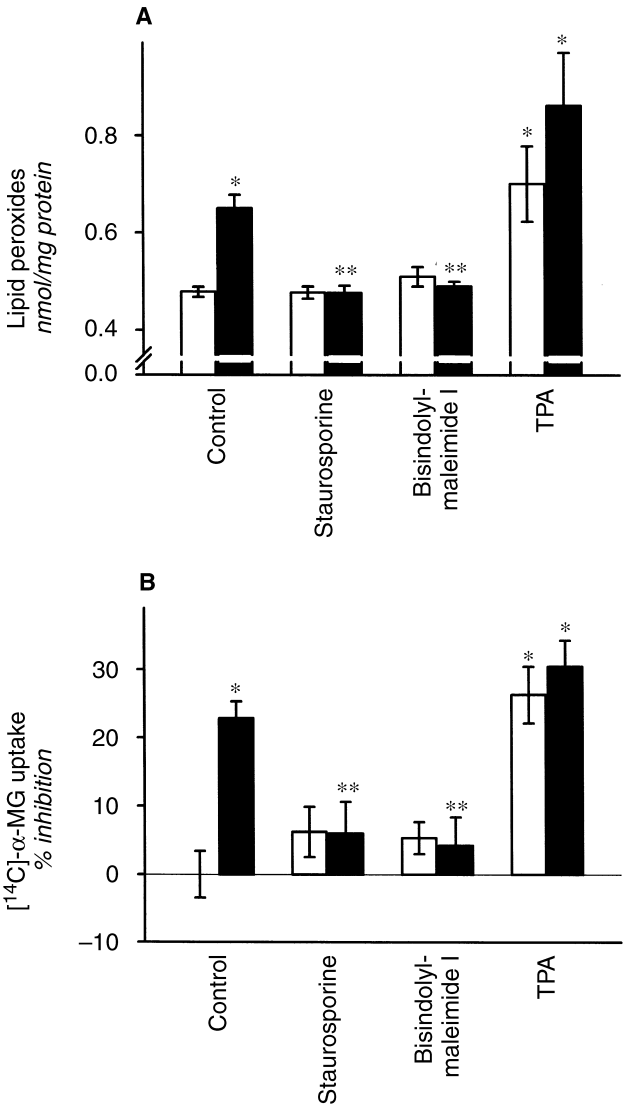

Fig. 6. Effects of 12-O-tetradecanoylphorbol-13-acetate (TPA) and staurosporine on 5 (□) or 25 (■) mmol/L glucose-induced lipid peroxides formation (*A*) and ^{14}C-α-MG uptake (*B*). PTCs were treated with staurosporine (10^{-7} mol/L) for 30 minutes prior to the treatment of 25 mmol/L glucose or were incubated with 25 mmol/L glucose alone or together with TPA for four hours. Values are the means ± SE of four independent experiments with triplicate dishes. *$P < 0.05$ vs. control; **$P < 0.05$ vs. 25 mmol/L glucose alone.

DISCUSSION

We document that high glucose induces inhibition of α-MG uptake through LPO formation, and that activation of PKC may play a role in high-glucose–induced LPO formation in primary cultured rabbit renal PTCs. Even though glucose-induced inhibition of α-MG uptake and stimulation of LPO formation were reported in a variety of cell types and tissues [4, 25, 26], direct evidence demonstrating the relationship between SGLT and LPO formation is not yet available. Hyperglycemia may increase the generation of free radicals in many ways, such

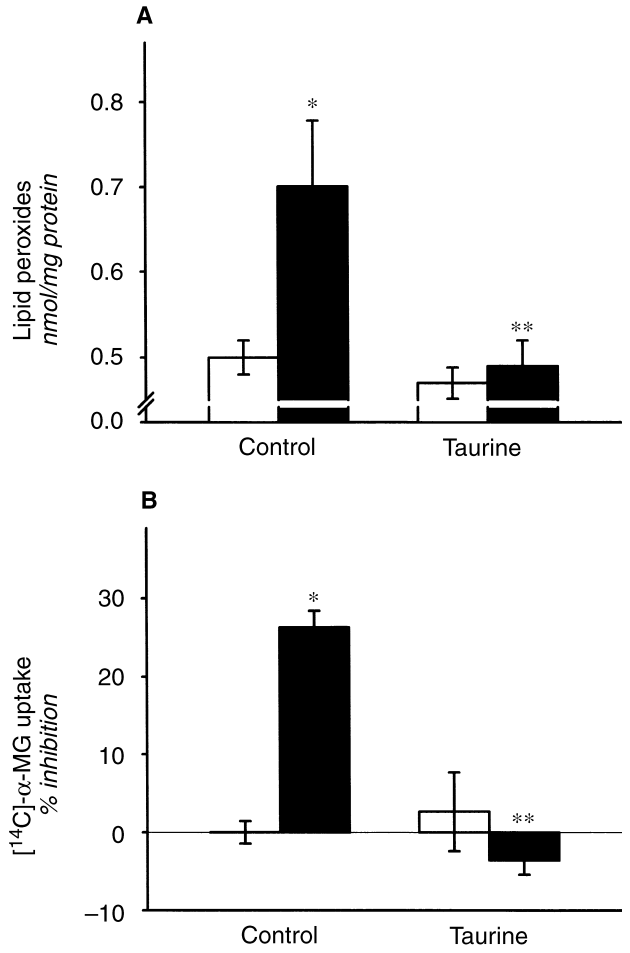

Fig. 7. Effects taurine on 5 mmol/L glucose (□) or 12-O-tetradecanoylphorbol-13-acetate (TPA) (■)-induced lipid peroxides formation (*A*) and ^{14}C-α-MG uptake (*B*). PTCs were treated with taurine (2 mmol/L) for 30 minutes prior to the treatment of TPA (100 ng/mL) for four hours. Values are the means ± SE of four independent experiments with triplicate dishes. *$P < 0.05$ vs. control; **$P < 0.05$ vs. 25 mmol/L glucose alone.

as glucose autoxidation, auto-oxidative glycosylation (glycoxidation), increased polyol pathway metabolism with subsequent "pseudohypoxia," and decreased natural antioxidant defenses [27]. Generation of different kinds of reactive oxygen radicals can oxidize membrane lipids or proteins and inactive enzymes, which can impair cellular transport and function and lead to cell death [28, 29]. In the present study, the rate of α-MG uptake was observed to be lower in monolayers treated with high glucose than that of monolayers maintained with normal glucose concentration. This result is consistent with observations in LLC-PK$_1$ cells [13]. Oxygen radicals also inhibited SGLTs of plasma membrane vesicle of LLC-PK$_1$ cells. Several lines of evidence also suggest that increased generation of reactive oxygen metabolites such as superoxide anion and hydrogen peroxides occurred in diabetes mellitus in association with hyperglycemia [30, 31]. In our current study, high glucose inhibited α-MG uptake and increased LPO formation. The relationship between LPO formation and α-MG uptake, especially in the constancy of time course, is not certain. However, based on other reports [32, 33], it is possible that a direct oxidative attack on glucose transporter protein, a significant proportion appeared secondary to the derangement in membrane physical properties that accompanied oxidative damage, and the alteration of paracellular and transcellular pathway is complicated in the inhibition of α-MG uptake. Furthermore, we clearly demonstrated that high glucose can increase the levels of LPO in PTCs as a result of glucose-induced oxidative stress and taurine, which is an endogenous antioxidant in the kidney [34, 35], blocked high-glucose–induced LPO formation and α-MG uptake inhibition. However, mannitol did not affect α-MG uptake and LPO production. In addition, high glucose did not affect the trypan blue exclusion test, as compared with control glucose. These results suggest that high-glucose–induced inhibition of α-MG uptake and LPO formation increase are not affected by osmolarity or cell viability changes, although it does not seem to exclude any pathological role of increased LPO.

Enhanced PKC signaling in high glucose is implicated in the complications of diabetes, including nephropathy [36, 37]. These results showed that the inhibition of the α-MG uptake induced by a high glucose level is mimicked by TPA and restored to normal by staurosporine, PKC inhibitor, thus suggesting PKC-mediated inhibition of α-MG uptake. The mechanism responsible for PKC activation by a high glucose level has been considered to be due to an increase in the DAG level, a physiological activator of PKC [38–40]. High glucose increases the de novo synthesis of DAG. Taken together, these data suggest that a high glucose level may inhibit the α-MG uptake by phosphorylating SGLTs directly via the activation of PKC in PTCs [41]. This hypothesis is evidenced by the report that rabbit renal SGLT1 has a PKC binding site and is modulated by PKC [42]. On the other hand, activation of PKC reduced the transport rates of rabbit and rat SGLT1, but increased the transport rate of human SGLT1. Therefore, the effect of protein kinases depends critically on the transporter and the isoform being expressed [42].

Interestingly, hyperglycemia-induced oxidative stress may mediate the adverse effects of PKC-β isoforms by the activation of the DAG-PKC pathway, because treatment with D-α-tocopherol, an antioxidant, was able to prevent many glucose-induced vascular dysfunctions and inhibit DAG-PKC activation [43]. Thus, it is possible that some of the PKC activation induced by high glucose could be the result of excessive oxidants, which are known to activate PKC. However, we showed that staurosporine strongly suppressed LPO formation in PTCs

exposed to high glucose, suggesting the role of PKC in high-glucose–induced increase of oxidative stress. Ha and Endou reported that glomerulus and proximal tubules are the most vulnerable sites of LPO formation, and PKC stimulates LPO formation [44]. Because TPA inhibited α-MG uptake and increased LPO formation and because staurosporine and bisindolylmaleimide I prevented high-glucose–induced inhibition of α-MG uptake and an increase of LPO formation, and because taurine blocked TPA-induced α-MG uptake and an increase of LPO formation in PTCs, it is hypothesized that there might be a close relationship between α-MG uptake and LPO formation via PKC in the PTCs exposed to high glucose for four hours. This possibility is supported by a report that activation of PKC may play a role in early phase of high-glucose–induced glomerular LPO formation [4]. Probably mechanisms other than PKC activation could have role in the glucose-induced inhibition of α-MG uptake. Therefore, our findings may provide new insights into the pathophysiological mechanisms of SGLT's dysfunction in diabetes mellitus. In conclusion, high glucose inhibits, in part, Na^+/glucose cotransporter activity through LPO formation in PTCs, and PKC appears to be involved in this action.

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